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Stephen L. Mikkelsen, Captain, USAF
Identification of Substances Interfering With Illicit Drug Testing
1988, 40 pages, Master of Science, University of Utah

ABSTRACT

In response to intensified urine testing for illicit drugs, drug users have attempted to falsify results by several schemes including in vitro adulteration of specimens. Additives that were claimed to invalidate enzyme immunoassay (EIA) drug assays were investigated. An investigation was also undertaken to determine whether adulterated urines could be identified so they might be rejected.

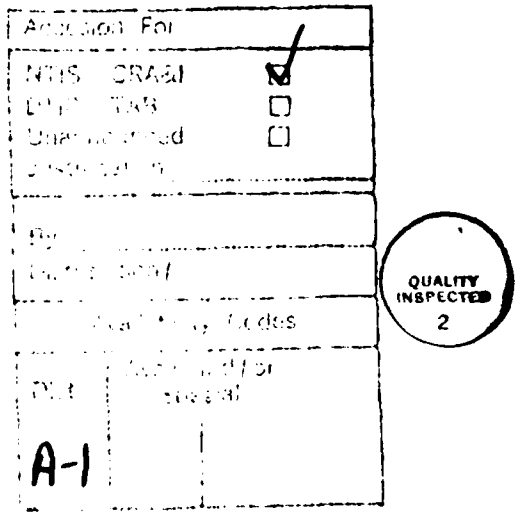
Adulterants were added at several concentrations to 222 EIA positive specimens confirmed by gas chromotography and mass spectrometry (GC/MS) for illicit drugs. Specimens were reanalyzed by the EIA screening procedures using a Hitachi 704 analyzer.

At the highest concentration evaluated, the adulterants (NaCl, Visine, Vestal medicated liquid handsoap, liquid Drano, liquid Chlorox bleach, Heinz vinegar, golden seal tea, and Real Lemon concentrated lemon juice) interfered with the drug assays differently. Amphetamine assays were affected by NaCl, Drano and bleach. Barbiturate assays were affected by liquid handsoap, Drano and bleach. Benzodiazepine assays were affected by Visine, liquid handsoap, Drano and bleach. Cocaine assays were affected by NaCl, Drano and bleach. Opiate assays were

affected by NaCl, Drano and bleach. The marijuana assays were affected by all except the lemon juice. The assays were unaffected at lower concentrations.

To identify adulterated urines, we monitored pH, relative density, urine color and turbidity at adulterant levels which falsified the EIA results. Specimens contaminated with NaCl had specific gravities greater than 1.035. Liquid Drano, bleach and vinegar produced a urine pH outside of the physiological range. Golden seal tea caused a dark appearance, and specimens containing liquid soap were unusually cloudy. Lemon juice had no effect on the assays. Visine was the only adulterant not detected.

Because EIA can be invalidated by specimen adulteration, drug testing should include assessment of pH, specific gravity and appearance. Suspect specimens should be rejected. Because not all adulterants can be detected, observed collection is recommended.



IDENTIFICATION OF SUBSTANCES INTERFERING WITH ILLICIT DRUG TESTING

by

Stephen L. Mikkelsen

A thesis submitted to the faculty of
The University of Utah
in partial fulfillment of the requirements for the degree of

Master of Science

in

Medical Laboratory Science

Department of Pathology

The University of Utah

June 1988

THE UNIVERSITY OF UTAH GRADUATE SCHOOL

SUPERVISORY COMMITTEE APPROVAL

of a thesis submitted by

Stephen L. Mikkelsen

This thesis has been read by each member of the following supervisory committee and by majority vote has been found to be satisfactory.

April 25, 1988 — Chair: K. Owen Ash

April 25, 1988 — Lee K. Roberts

April 25, 1988 — Joseph C. Veltri

THE UNIVERSITY OF UTAH GRADUATE SCHOOL

FINAL READING APPROVAL

To the Graduate Council of the University of Utah:

I have read the thesis of Stephen L. Mikkelsen **in its final form and have found that (1) its format, citations, and bibliographic style are consistent and acceptable; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the Supervisory Committee and is ready for submission to the Graduate School.**

April 25, 1988
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ABSTRACT

In response to intensified urine testing for illicit drugs, drug users have attempted to falsify results by several schemes including in vitro adulteration of specimens. Additives that were claimed to invalidate enzyme immunoassay (EIA) drug assays were investigated. An investigation was also undertaken to determine whether adulterated urines could be identified so they might be rejected.

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To identify adulterated urines, we monitored pH, relative density, urine color and turbidity at adulterant levels which falsified the EIA results. Specimens contaminated with NaCl had specific gravities greater than 1.035. Liquid Drano, bleach and vinegar produced a urine pH outside of the physiological range. Golden seal tea caused a dark appearance, and specimens containing liquid soap were unusually cloudy. Lemon juice had no effect on the assays. Visine was the only adulterant not detected.

Because EIA can be invalidated by specimen adulteration, drug testing should include assessment of pH, specific gravity and appearance. Suspect specimens should be rejected. Because not all adulterants can be detected, observed collection is recommended.

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ACKNOWLEDGMENTS

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INTRODUCTION

In the past five years, a growing public concern over the use of illicit drugs in the workplace has led to an interest in urinalysis as a way to detect and deter drug use (1). Testing for illicit drugs in urine has been suggested and, in many cases, implemented for prospective and current employees in industry; for personnel of the armed forces; for parolees and bail seekers in civilian court systems; for workers in the transportation industry; and for individuals who serve as role models, such as atheletes (2). Two factors have led to the widespread use of urinalysis for drugs: technical developments in testing methods (e.g., the development of the Syva EMIT d.a.u. procedures) (3) and the growing demand for drug testing by industry (4). Society is becoming increasingly aware of the negative impact of drug use on public safety and of the financial impact on industry of drug abuse related absence, decreased safety and lost productivity. The reported annual cost of productivity loss and health care claims of employees who abuse drugs has been estimated at $33 billion in the United States (3).

Effective programs for the detection of illicit drugs in human urine specimens are best accomplished with sensitive drug testing procedures. Because of the numerous

legal features of drug detection programs (3), the analytical results must be unquestionably reliable and able to withstand vigorous legal scrutiny.

Drug testing laboratories are being required to develop extensive systems to produce results that are secure from false positives and defensible in a forum where the data may be challenged (e.g., a legal hearing) (3). Adequate methods are not in place, however, to secure the data from false negatives. Because the most commonly used drug screening assay, the enzyme immunoassay (EIA), is being used by most clinical laboratories, it is important to understand the assay limitations, such as specimen pH range, specific gravity, and ionic strength (5). An interferent addition that might cause the urine to be outside these limitations would produce an invalid test. Several methods of interference which have been claimed to produce false negative results (e.g., by addition of a foreign substance) are common knowledge to many individuals who undergo urine testing for illicit drugs (6-9).

The aim of this study is to investigate the ability of certain commonly available substances to cause false negative results when introduced into a urine specimen that would otherwise test positive by EIA, so as to render the enzyme immunoassay drug testing methodology ineffective. This would result in erroneous reporting of a positive urine specimen as negative for illicit drugs.

Because those involved in illicit drug testing are usually required to provide a urine sample with little or no advance notice, they have little opportunity to implement in vivo manipulation procedures. Therefore, this study was limited to in vitro urine adulteration.

After conducting a literature search and interviews with admitted drug abusers, drug abuse treatment center personnel and clinical toxicologists, eight different substances were identified as additives being used by drug users to contaminate their urine specimens so as to avoid the detection of illicit drugs. Suspected interferents so far identified that are currently being utilized and discussed within the drug community are adulterations with household vinegar (6), table salt (6), liquid laundry bleach (6), concentrated lemon juice (7), caustic household cleansers (7), health foods such as golden seal (8), liquid handsoap from restroom dispensers (9), and Visine eyedrops. In the studies reported herein, these substances were introduced into EIA-positive urine specimens which had also been confirmed positive by gas chromotography and mass spectrometry (GC/MS) to determine their actual effect on the EIA urine drug testing results.

By identifying a list of commonly used interferents to manipulate urine specimens, and by verifying that false negative results are indeed obtained by the use of these interferents, drug testing laboratories can be educated to the fact that specimen adulteration can adversely affect

test results. Specimen adulteration is a topic openly discussed in drug literature circulated by illicit drug users, but not adequately reported in laboratory journals. It is important that the drug testing laboratories be informed.

This study also attempted to identify an effective means of detecting urine specimens that have been contaminated with a foreign substance so that another urine specimen may be obtained which will not interfere with the enzyme immunoassay technique of drug detection. This will improve the validity of illicit drug testing results.

MATERIALS AND METHODS

Materials

Morphine sulfate (100 mg/dl), benzoyl ecognine (100 mg/dl), and 11-nor-delta-9-THC-9-COOH (10 ng/ul) were obtained from Alltech Associated Applied Science, Deerfield, Illinois. Amphetamine sulfate (100 mg/dl) was obtained from Smith Kline, Philadelphia, Pennsylvania. Oxazepam (100 mg/dl) was obtained from Wyeth Laboratories, Philadelphia, Pennsylvania. Secobarbital was obtained from Eli Lilly and Company, Indianapolis, Indiana. For the semiquantitative enzyme immunoassays, the assay reagents (EMIT d.a.u.) and calibrators were obtained from the Syva Company. The instrument used for the EIA analysis was the Hitachi 704 Auto Analyzer from Boehringer Mannheim Diagnostics, Indianapolis, Indiana. The pH meter used was the Beckman Expandomatic SS-2. The refractometer used for specific gravity determinations was the Reichert TS meter. The eight interferents investigated in this project were all purchased from a local supermarket. Aliquots (5 ml) from 222 EIA positive and GC/MS-confirmed positive urine specimens were obtained from Associated Regional and University Pathologists (ARUP).

Spiked Urine Preparation

Purified drugs (metabolite or standards) obtained from the respective commercial manufacturers were added to 10 ml aliquots of urine from a healthy drug-free volunteer. These spiked aliquots contained a final concentration of 1.0 ug/ml of amphetamine, secobarbital, oxazepam, benzoyl ecgonine (cocaine), morphine, or 120 ng/ml of 11-nor-delta-9-THC-9-COOH (cannabinoid), as shown in Table 1. These concentrations are somewhat higher than twice the cut-off values for a positive result. The "positive" cut-off values for amphetamines, barbiturates, cocaine, benzodiazepines and opiates are 0.3 ug/ml. For marijuana, the "positive" cut-off is 50 ng/ml. Thus a 1:1 mixture of spiked urine and interferent will provide for drug concentrations at 0.5 ug/ml and 60 ng/ml, well above the sensitivity of the test. Aliquots of spiked urine samples were diluted 1:1 with normal saline (0.85% NaCl) and assayed to confirm that EIA positive results were obtained on the dilutions prior to testing the interferents.

Interferent Preparation

Prior to mixing with the spiked urine samples, the interferents (e.g., liquid Chlorox bleach, household Heinz vinegar, Vestal medicated liquid handsoap, liquid Drano, Visine eye drops, Real Lemon concentrated lemon juice, Morton's table salt, and Natural Brand golden seal) were placed into concentrations thought to adversely affect

Table 1

Spiked-urine Preparation

Drug	Starting Concentration	Amount Added to 10 ml Urine	Final Concentration
Amphetamine sulfate	100 mg/dl	10 ul	1.0 ug/ml
Benzoylecgonine	100 mg/dl	10 ul	1.0 ug/ml
Secobarbital	100 mg/dl	10 ul	1.0 ug/ml
Oxazepam	100 mg/dl	10 ul	1.0 ug/ml
Morphine sulfate	100 mg/dl	10 ul	1.0 ug/ml
11-nor-9-THC-9-COOH	10 ng/ul	120 ul	120 ng/ml

drug testing results (5, 9, 10). Normal saline (0.85% NaCl) was used as a diluent.

The golden seal was prepared into a tea by dissolving 120 mg of golden seal (ground leaves and stem) in 1.0 ml of normal saline at 37°C. The tea was covered and allowed to sit overnight at 4°C. The following day the tea was filtered to remove any undissolved plant residue. The golden seal tea was then aliquoted and placed into concentrations ranging from 10 mg/ml to 50 mg/ml.

It has been reported that salt concentrations of greater than 50 mg/ml (10), commercial soap concentrations of greater than 1 ml/dl (9), and solutions that change the urine pH to less than 5 or greater than 8 (5) can produce a false negative result with the Syva EMIT test.

After these starting concentrations had been determined, serial dilutions of the interferent concentrations were made and added to fixed concentrations of spiked urine in order to determine the minimum amount of interferent needed to produce a false negative result.

Standard Enzyme Assay

The assay technique utilized in the performance of the urine drug testing for this study was an enzyme immunoassay (11). Because of its cost effectiveness, the assay most commonly used in the United States is the EMIT d.a.u. manufactured by the Syva Company (7). For this reason, the EMIT d.a.u., a system designed for use in laboratories with

large sample throughput was used in conjunction with a Hitachi 704 chemistry analyzer.

The EMIT d.a.u. is a homogeneous enzyme immunoassay technique used for the microanalysis of specific compounds in biological fluids. In the performance of an EMIT assay, urine is mixed with two reagents: Reagent A contains antibodies to the drug, the coenzyme nicotinamide adenine dinucleotide (NAD), and the substrate glucose-6-phosphate (G-6-P); Reagent B contains a drug derivative labeled with the enzyme glucose-6-phosphate dehydrogenase (G6P-DH). Reagent A is added to the sample first, and the antibody binds to the drug it recognizes. Reagent B is added next, and the enzyme-labeled drug binds with any remaining drug antibodies; this binding decreases the enzyme activity. Some enzyme remains unbound and therefore stays active in the reaction mixture. This residual enzyme activity is directly proportional to the concentration of drug in the sample. The active enzyme converts NAD to NADH, resulting in an absorbance change that is measured spectrophometrically at 340 nm. Bacterial (*Leuconostoc mesenteroides*) G6P-DH is used in this assay (11). Specimens for testing were prepared by adding 100 ul of each drug-spiked urine to 100 ul of each of the interferent concentrations. The tubes were vortexed (mixed) and allowed to sit two hours at room temperature in order to equilibrate prior to use. These samples were then analyzed on the Hitachi 704 with

the Syva EMIT d.a.u. assays for amphetamines, barbiturates, benzodiazepine, cocaine, opiates, and cannabinoids.

Positive commercial controls and negative controls (drug-free urine) were run simultaneously to verify proper instrument operation and reporting. After the mixtures of drug-spiked urine and interferents were assayed, mixtures of interferent concentrations and urine specimens confirmed positive by GC/MS for illicit drug metabolites were assayed.

The suspected interferents outwardly seemed to incorporate pH, ionic strength and specific gravity changes to invalidate the testing (12). In order to verify this, the pH and specific gravity of each sample were obtained prior to the analysis.

RESULTS

Analysis of Drug-Spiked Urine

The drug-free urine aliquots spiked with 1.0 ug/ml (120 ng/ml of THC) of drug metabolites were assayed on the Hitachi 704 to confirm positive results using the Syva EMIT d.a.u. assay method. Results higher than the positive cut-off values (50 ng/ml for THC and 0.3 ug/ml for amphetamines, barbiturates, benzodiazepines, cocaine and opiates) were obtained from each aliquot of drug-spiked urine.

Next, the concentrations of the interferents required to change the spiked urines' positive results to false negative results were determined by adding increasing amounts of the suspected interferents to fixed volumes (1 ml) of normal saline (0.85% NaCl). The interferent concentrations were then mixed 1:1 with the metabolite-spiked urines to find which concentrations produced false negative results, and at which concentrations this would occur. False negative results were obtained when the following final concentrations of interferents were present in the spiked urines:

NaCl	- 50 mg NaCl/ml spiked urine
vinegar	- 85 ul vinegar/ml spiked urine
liquid bleach	- 12 ul bleach/ml spiked urine
liquid Drano	- 12 ul Drano/ml spiked urine

liquid handsoap	- 12 ul handsoap/ml spiked urine
Visine eye drops	- 50 ul Visine/ml spiked urine
lemon juice concentrate	- 500 ul lemon juice/ml spiked urine
golden seal tea	- 15 mg golden seal/ml spiked urine

Having determined the concentrations of interferents that caused false negative results in spiked urines, these concentrations served as starting concentrations to be mixed with EIA positive and GC/MS-confirmed positive urine specimens in order to try to create false negative results in actual specimens containing representative drug metabolite concentrations.

Effect of Adulterants on Detection of Amphetamines

Forty urine specimens previously confirmed positive for amphetamines by GC/MS were reassayed to obtain baseline absorbance values and estimate the concentration of amphetamines in each specimen. These assays were conducted on 100 ul aliquots of positive urine mixed with 100 ul of drug-free urine. Absorbance readings were obtained and then plotted on semilogarithmic graph paper (absorbance versus concentration). The semiquantitative assessment of the amphetamines in each urine specimen was then determined. The amphetamine concentrations of the 40 specimens ranged from a low of 0.34 ug/ml to a high of 4.72 ug/ml.

Urinalysis including pH, specific gravity, urine color and turbidity was performed on each of the 40 urine specimens prior to the addition of any interferents. Each of the 40 urine specimens was then aliquoted into 100 ul portions. To each 100 ul aliquot was added 100 ul of varying concentrations of the eight different interferents investigated.

The mixtures of positive urine and interferents were then analyzed on the Hitachi 704 Auto Analyzer. The results are summarized in Table 2. Urine samples containing an estimated amphetamine concentration of up to 1.42 ug/ml were falsely negative with NaCl concentrations of 75 mg/ml urine. Urine samples containing an estimated amphetamine concentration of up to 0.52 ug/ml were reported as negative with liquid bleach or Drano in concentrations of 12 ul/ml urine. For estimated amphetamine concentrations of up to 1.80 ug/ml, liquid bleach or Drano concentrations of 23 ul/ml urine were required. For estimated amphetamine concentrations of up to 4.50 ug/ml, the liquid bleach or Drano concentrations required were 42 ul/ml urine. The estimated amphetamine concentration for a false negative result with a mixture of either liquid bleach or Drano could be extended to 4.65 ug/ml with the bleach or Drano addition in a concentration of 125 ul/ml urine. No effective interferent concentrations of the other interferents under investigation were found that would enable us to change the positive results to false negative. The

Table 2

Amphetamine Results of GC/MS-confirmed Positive Urine Specimens
Mixed with Interferents

Estimated Drug Concentration (ug/ml)	NaCl	Visine	Soap	Bleach Drano	Gold Seal	Vinegar	Lemon Juice
	75 mg/ml	(*)	(*)	12 ul/ml	(*)	(*)	(*)
.34	-	+	+	-	+	+	+
.35	-	+	+	-	+	+	+
.35	-	+	+	-	+	+	+
.35	-	+	+	-	+	+	+
.52	-	+	+	-	+	+	+
				23 ul/ml			
.64	-	+	+	-	+	+	+
.83	-	+	+	-	+	+	+
.92	-	+	+	-	+	+	+
1.06	-	+	+	-	+	+	+
1.10	-	+	+	-	+	+	+
1.10	-	+	+	-	+	+	+
1.22	-	+	+	-	+	+	+
1.42	-	+	+	-	+	+	+
	(*)						
1.80	+	+	+	-	+	+	+
1.80	+	+	+	-	+	+	+
				42 ul/ml			
2.00	+	+	+	-	+	+	+
2.08	+	+	+	-	+	+	+
2.20	+	+	+	-	+	+	+
2.30	+	+	+	-	+	+	+
2.38	+	+	+	-	+	+	+
2.55	+	+	+	-	+	+	+

Table 2 (Continued)

Estimated Drug Concentration (ug/ml)	NaCl	Visine	Soap	Bleach Drano	Gold Seal	Vinegar	Lemon Juice
2.60	+	+	+	-	+	+	+
2.80	+	+	+	-	+	+	+
2.84	+	+	+	-	+	+	+
3.20	+	+	+	-	+	+	+
3.23	+	+	+	-	+	+	+
3.25	+	+	+	-	+	+	+
3.45	+	+	+	-	+	+	+
3.45	+	+	+	-	+	+	+
4.20	+	+	+	-	+	+	+
4.22	+	+	+	-	+	+	+
4.22	+	+	+	-	+	+	+
4.50	+	+	+	- 125 ul/ml	+	+	+
4.52	+	+	+	-	+	+	+
4.52	+	+	+	-	+	+	+
4.64	+	+	+	-	+	+	+
4.65	+	+	+	- (*)	+	+	+
4.72	+	+	+	+	+	+	+

(*) No effective interferent concentration found
- False negative EIA result
+ Positive EIA result

concentrated lemon juice added in vitro to the EIA positive and GC/MS-confirmed positive urines had no effect on any of the assay results, regardless of the levels introduced.

Urinalysis was repeated on the adulterated urine specimens that changed from testing positive to negative. The pH, specific gravity, urine color and turbidity were again recorded. A comparison of the pre- and post-urinalysis results has been presented at the end of this section.

Effect of Adulterants on Detection of Barbiturates

Twenty urine specimens previously confirmed positive for barbiturates by GC/MS were reassayed to obtain baseline absorbance values and estimate the concentration of barbiturates in each specimen. These assays were conducted on 100 ul aliquots of positive urine mixed with 100 ul of drug-free urine. Absorbance readings were obtained and then plotted on semilogarithmic graph paper (absorbance versus concentration). The semiquantitative concentration of the amount of barbiturates in each urine specimen was then determined. The barbiturate concentrations of the 20 specimens ranged from a low of 0.38 ug/ml to a high of 2.90 ug/ml.

Urinalysis was conducted on each of the 20 urine specimens before and after addition of the interferents. The pH, specific gravity, urine color and turbidity were

recorded. The data comparison is discussed later in this section.

Each of the 20 urine specimens was then aliquoted into 100 ul portions. To each 100 ul aliquot was added 100 ul of varying concentrations of the eight different interferents.

The mixtures of positive urine and interferents were then analyzed on the Hitachi 704 Auto Analyzer. The results are summarized in Table 3. Urine samples containing an estimated barbiturate concentration of up to 0.38 ug/ml were falsely negative with NaCl concentrations of 75 mg/ml urine. Urine samples containing an estimated barbiturate concentration of up to 0.38 ug/ml were reported as negative with a liquid handsoap concentration of 23 ul/ml urine, and with liquid handsoap concentrations of 107 ul/ml urine, the estimated barbiturate concentrations showing a false negative result could be extended to 1.10 ug/ml Liquid bleach and Drano also affected this assay. Urine samples containing an estimated barbiturate concentration of up to 0.38 ug/ml were reported as negative when bleach or Drano concentrations of 23 ul/ml urine were added, and with bleach or Drano concentrations of 125 ul/ml urine, the estimated barbiturate concentrations showing a false negative result could be extended to 1.10 ug/ml. No effective concentrations of the other interferents under investigation were found that would enable us to change the positive results to falsely negative.

Table 3

Barbiturate Results of GC/MS-confirmed Positive Urine Specimens Mixed with Interferents

Estimated Drug Concentration (ug/ml)	NaCl	Visine	Soap	Bleach Drano	Gold Seal	Vinegar	Lemon Juice
	75 mg/ml	(*)	23 ul/ml	23 ul/ml	(*)	(*)	(*)
.38	-	+	-	-	+	+	+
	(*)		107 ul/ml	125 ul/ml			
.62	+	+	-	-	+	+	+
.66	+	+	-	-	+	+	+
.68	+	+	-	-	+	+	+
.70	+	+	-	-	+	+	+
1.05	+	+	-	-	+	+	+
1.10	+	+	-	-	+	+	+
1.10	+	+	-	-	+	+	+
			(*)	(*)			
1.45	+	+	+	+	+	+	+
1.55	+	+	+	+	+	+	+
1.65	+	+	+	+	+	+	+
1.78	+	+	+	+	+	+	+
1.80	+	+	+	+	+	+	+
1.90	+	+	+	+	+	+	+
1.98	+	+	+	+	+	+	+
2.10	+	+	+	+	+	+	+
2.38	+	+	+	+	+	+	+
2.70	+	+	+	+	+	+	+
2.70	+	+	+	+	+	+	+
2.90	+	+	+	+	+	+	+

(*) No effective interferent concentration found
- False negative EIA result
+ Positive EIA result

Effect of Adulterants on Detection of Benzodiazepines

Forty urine specimens previously confirmed positive for benzodiazepines by GC/MS were reassayed to obtain baseline absorbance values and estimate the concentration of benzodiazepines in each specimen. These assays were conducted on 100 ul aliquots of positive urine mixed with 100 ul of drug-free urine. Absorbance readings were obtained and then plotted on semilogarithmic graph paper (absorbance versus concentration). The semiquantitative concentration of the amount of benzodiazepines in each urine specimen was then determined. The benzodiazepine concentrations of the 40 specimens ranged from a low of 0.38 ug/ml to a high of >6.50 ug/ml.

Urinalysis was then conducted on each of the 40 urine specimens before and after addition of the interferents. The pH, specific gravity, urine color and turbidity were recorded. The data comparison is discussed later in this section.

Each of the 40 urine specimens was then aliquoted into 100 ul portions. To each 100 ul aliquot was added 100 ul of varying concentrations of the eight different interferents.

The mixtures of positive urine and interferents were then analyzed on the Hitachi 704 Auto Analyzer. The results are summarized in Table 4. Urine containing an estimated benzodiazepine concentration of up to 0.78 ug/ml was falsely negative with Visine concentrations of 107

Table 4

Benzodiazepine Results of GC/MS-confirmed Positive Urine Specimens Mixed with Interferents

Estimated Drug Concentration (ug/ml)	NaCl	Visine	Soap	Bleach Drano	Gold Seal	Vinegar	Lemon Juice
	(*)	107 ul/ml	42 ul/ml	125 ul/ml	(*)	(*)	(*)
.38	+	-	-	-	+	+	+
.53	+	-	-	-	+	+	+
.62	+	-	-	-	+	+	+
.64	+	-	-	-	+	+	+
.64	+	-	-	-	+	+	+
.65	+	-	-	-	+	+	+
.66	+	-	-	-	+	+	+
.78	+	- (*)	-	-	+	+	+
.90	+	+	-	-	+	+	+
.92	+	+	-	-	+	+	+
.92	+	+	-	-	+	+	+
1.00	+	+	-	-	+	+	+
1.10	+	+	-	-	+	+	+
1.13	+	+	-	-	+	+	+
1.14	+	+	-	-	+	+	+
1.16	+	+	-	-	+	+	+
1.18	+	+	-	-	+	+	+
1.20	+	+	-	-	+	+	+
1.22	+	+	-	-	+	+	+
1.47	+	+	-	-	+	+	+
1.66	+	+	-	-	+	+	+
1.84	+	+	-	-	+	+	+
1.84	+	+	-	-	+	+	+

Table 4 (Continued)

Estimated Drug Concentration (ug/ml)	NaCl	Visine	Soap	Bleach Drano	Gold Seal	Vinegar	Lemon Juice
2.14	+	+	-	-	+	+	+
2.14	+	+	-	-	+	+	+
2.40	+	+	-	-	+	+	+
2.42	+	+	-	-	+	+	+
2.42	+	+	-	-	+	+	+
2.56	+	+	-	-	+	+	+
2.56	+	+	-	-	+	+	+
				(*)			
3.00	+	+	-	+	+	+	+
3.00	+	+	-	+	+	+	+
3.82	+	+	-	+	+	+	+
4.20	+	+	-	+	+	+	+
5.90	+	+	-	+	+	+	+
6.10	+	+	-	+	+	+	+
6.20	+	+	-	+	+	+	+
			(*)				
>6.50	+	+	+	+	+	+	+
>6.50	+	+	+	+	+	+	+

(*) No effective interferent concentration found
\+ Positive EIA result
\- False negative EIA result

ul/ml of urine. Urine samples containing an estimated benzodiazepine concentration of up to 6.20 ug/ml were falsely negative with a liquid handsoap concentration of 42 ul/ml urine. Urine samples containing an estimated benzodiazepine concentration of up to 2.56 ug/ml were falsely negative with liquid bleach or Drano concentrations of 125 ul/ml urine. No effective concentrations of the other interferents were found that would enable us to change the positive results to negative.

Effect of Adulterants on Detection of Cocaine

Forty urine specimens previously confirmed for cocaine by GC/MS were reassayed to obtain baseline absorbance values and estimate the concentration of cocaine in each specimen. It is important to note that in determining cocaine concentration, that benzoyl ecgonine (a primary cocaine metabolite) concentration is what was actually being measured. For simplicity, the two terms in this paper will be used interchangeably. These assays were conducted on 100 ul aliquots of positive urine mixed with 100 ul of drug-free urine. Absorbance readings were obtained and then plotted on semilogarithmic graph paper (absorbance versus concentration). The semiquantitative concentration of the amount of cocaine in each urine specimen was then determined. The cocaine concentrations of the 40 specimens ranged from a low of 0.30 ug/ml to a high of >2.70 ug/ml.

Urinalysis was then conducted on each of the 40 urine specimens before and after addition of the interferents. The pH, specific gravity, urine color and turbidity were recorded. The data comparison is discussed later in this section.

Each of the 40 urine specimens was then aliquoted into 100 ul portions. To each 100 ul was added 100 ul of varying concentrations of the eight different interferents.

The mixtures of positive urine and interferents were then analyzed on the Hitachi 704 Auto Analyzer. The results are summarized in Table 5. Urine containing an estimated cocaine concentration of up to 1.18 ug/ml was falsely negative with NaCl concentrations of 75 mg/ml of urine. Urine samples containing an estimated cocaine concentration of up to 1.18 ug/ml were falsely negative with liquid bleach or Drano in concentrations of 42 ul/ml of urine. The estimated cocaine concentrations reported as falsely negative could be extended to 1.72 ug/ml with the addition of bleach or Drano at a concentration of 58 ul/ml of urine, and with a caustic concentration of 125 ul/ml of urine, the estimated cocaine concentration reported as negative could be extended even further to 1.82 ul/ml. No effective concentrations of the other interferents under investigation were found that would enable us to change the positive results to negative.

Table 5

Cocaine Results of GC/MS-confirmed Positive Urine Specimens Mixed with Interferents

Estimated Drug Concentration (ug/ml)	NaCl	Visine	Soap	Bleach Drano	Gold Seal	Vinegar	Lemon Juice
	75 mg/ml	(*)	(*)	42 ul/ml	(*)	(*)	(*)
.30	-	+	+	-	+	+	+
.30	-	+	+	-	+	+	+
.30	-	+	+	-	+	+	+
.30	-	+	+	-	+	+	+
.35	-	+	+	-	+	+	+
.36	-	+	+	-	+	+	+
.38	-	+	+	-	+	+	+
.43	-	+	+	-	+	+	+
.48	-	+	+	-	+	+	+
.58	-	+	+	-	+	+	+
.60	-	+	+	-	+	+	+
.75	-	+	+	-	+	+	+
.78	-	+	+	-	+	+	+
.80	-	+	+	-	+	+	+
.82	-	+	+	-	+	+	+
.85	-	+	+	-	+	+	+
.90	-	+	+	-	+	+	+
.91	-	+	+	-	+	+	+
.94	-	+	+	-	+	+	+
.96	-	+	+	-	+	+	+
.96	-	+	+	-	+	+	+
1.10	-	+	+	-	+	+	+
1.17	-	+	+	-	+	+	+
1.18	-	+	+	-	+	+	+

Table 5 (Continued)

Estimated Drug Concentration (ug/ml)	NaCl	Visine	Soap	Bleach Drano	Gold Seal	Vinegar	Lemon Juice
	(*)			58 ul/ml			
1.20	+	+	+	-	+	+	+
1.35	+	+	+	-	+	+	+
1.65	+	+	+	-	+	+	+
1.70	+	+	+	-	+	+	+
1.72	+	+	+	-	+	+	+
				125 ul/ml			
1.82	+	+	+	-	+	+	+
1.82	+	+	+	-	+	+	+
				(*)			
1.84	+	+	+	+	+	+	+
1.84	+	+	+	+	+	+	+
2.65	+	+	+	+	+	+	+
2.67	+	+	+	+	+	+	+
2.70	+	+	+	+	+	+	+
>2.70	+	+	+	+	+	+	+
>2.70	+	+	+	+	+	+	+
>2.70	+	+	+	+	+	+	+

(*) No effective interferent concentration found
- False negative EIA result
+ Positive EIA result

Effect of Adulterants on Detection of Opiates

Forty urine specimens previously confirmed positive for opiates by GC/MS were reassayed to obtain baseline absorbance values and estimate the concentration of opiates in each specimen. These assays were conducted on 100 ul aliquots of positive urine mixed with 100 ul of drug-free urine. Absorbance readings were obtained and then plotted on semilogarithmic graph paper (absorbance versus concentration). The semiquantitative concentration of the amount of opiates in each urine specimen was then determined. The opiate concentrations of the 40 specimens ranged from a low of 0.31 ug/ml to a high of >2.70 ug/ml.

Urinalysis was then conducted on each of the 40 urine specimens before and after addition of the interferents. The pH, specific gravity, urine color and turbidity were recorded. The data comparison is discussed later in this section.

Each of the 40 urine specimens was then aliquoted into 100 ul portions. To each 100 ul aliquot was added 100 ul of varying concentrations of the eight different interferents.

The mixtures of positive urine and interferents were then analyzed on the Hitachi 704 Auto Analyzer. The results are summarized in Table 6. Urine containing an estimated opiate concentration of up to 0.78 ug/ml was falsely negative with NaCl concentrations of 50 mg/ml of urine. Urine samples containing an estimated opiate

Table 6

Opiate Results of GC/MS-confirmed Positive Urine Specimens Mixed with Interferents

Estimated Drug Concentration (ug/ml)	NaCl	Visine	Soap	Bleach Drano	Gold Seal	Vinegar	Lemon Juice
	50 mg/ml	(*)	(*)	23 ul/ml	(*)	(*)	(*)
.31	-	+	+	-	+	+	+
.35	-	+	+	-	+	+	+
.38	-	+	+	-	+	+	+
.50	-	+	+	-	+	+	+
.74	-	+	+	-	+	+	+
.74	-	+	+	-	+	+	+
.78	-	+	+	-	+	+	+
	(*)						
1.10	+	+	+	-	+	+	+
1.15	+	+	+	-	+	+	+
1.24	+	+	+	-	+	+	+
1.40	+	+	+	-	+	+	+
1.40	+	+	+	-	+	+	+
1.40	+	+	+	-	+	+	+
1.40	+	+	+	-	+	+	+
1.50	+	+	+	-	+	+	+
1.55	+	+	+	-	+	+	+
1.55	+	+	+	-	+	+	+
1.65	+	+	+	-	+	+	+
2.10	+	+	+	-	+	+	+
2.14	+	+	+	-	+	+	+
2.32	+	+	+	-	+	+	+
2.36	+	+	+	-	+	+	+
				42 ul/ml			

Table 6 (Continued)

Estimated Drug Concentration (ug/ml)	NaCl	Visine	Soap	Bleach Drano	Gold Seal	Vinegar	Lemon Juice
2.40	+	+	+	-	+	+	+
2.55	+	+	+	-	+	+	+
2.68	+	+	+	-	+	+	+
2.68	+	+	+	-	+	+	+
2.68	+	+	+	-	+	+	+
2.68	+	+	+	-	+	+	+
2.68	+	+	+	-	+	+	+
2.69	+	+	+	-	+	+	+
2.69	+	+	+	-	+	+	+
2.69	+	+	+	-	+	+	+
2.70	+	+	+	- 125 ul/ml	+	+	+
2.70	+	+	+	-	+	+	+
2.70	+	+	+	- (*)	+	+	+
>2.70	+	+	+	+	+	+	+
>2.70	+	+	+	+	+	+	+
>2.70	+	+	+	+	+	+	+
>2.70	+	+	+	+	+	+	+
>2.70	+	+	+	+	+	+	+

(*) No effective interferent concentration found
- False negative EIA result
+ Positive EIA result

concentration of up to 2.36 ug/ml were reported as negative when liquid bleach or Drano concentrations of 23 ul/ml of saline was added. The estimated opiate concentration of up to 2.70 ug/ml was falsely negative with bleach or Drano concentrations of 42 ul/ml of urine. Two samples containing an estimated opiate concentration of 2.70 ug/ml required a bleach or Drano concentration of 125 ul/ml of urine. No effective concentrations of the other interferents under investigation were found that would enable us to change the positive results to negative.

Effect of Adulterants on Detection of Marijuana

Forty-two urine specimens previously confirmed positive for marijuana by GC/MS were reassayed to obtain baseline absorbance values and estimate the concentration of THC in each specimen. It is important to note that the THC concentration measured is actually the 11-nor-delta-9-THC-9-COOH (cannabinoid metabolite) concentration. For simplicity, the term THC will be used when describing marijuana concentrations. These assays were conducted on 100 ul aliquots of positive urine mixed with 100 ul of drug-free urine. Absorbance readings were obtained and then plotted on semilogarithmic graph paper (absorbance versus concentration). The THC concentrations of the 42 specimens ranged from a low of 31 ng/ml to a high of 122 ng/ml.

Urinalysis was then conducted on each of the 42 urine specimens before and after addition of the interferents. The pH, specific gravity, urine color and turbidity were recorded. The data comparison is discussed later in this section.

Each of the 42 urine specimens was then aliquoted into 100 ul portions. To each 100 ul aliquot was added 100 ul of varying concentrations of the eight different interferents.

The mixtures of positive urine and interferents were then analyzed on the Hitachi 704 Auto Analyzer. The results are summarized in Table 7. Urine containing an estimated THC concentration of up to 80 ng/ml was falsely negative with NaCl concentrations of 25 mg/ml urine and the estimated THC concentration could be extended up to 122 ng/ml with the addition of NaCl concentrations of 50 mg/ml of urine. Urine specimens containing an estimated THC concentration of up to 122 ng/ml were falsely negative with the addition of Visine, liquid handsoap, or bleach/Drano in the following concentrations: 125 ul Visine/ml of urine; 12 ul liquid handsoap/ml of urine; and 12 ul bleach or Drano/ml of urine, respectively. Golden seal tea in a concentration of 15 mg/ml of urine would produce false negative results in estimated THC concentrations of up to 61 ng/ml. The estimated THC concentration range of up to 122 ng/ml would produce negative results with a golden seal tea concentration of 30 mg/ml of urine. Vinegar in

Table 7

Marijuana Results of GC/MS-confirmed Positive Urine Specimens Mixed with Interferents

Estimated Drug Concentration (ng/ml)	NaCl	Visine	Soap	Bleach Drano	Gold Seal	Vinegar	Lemon Juice
	25 mg/ml	125 ul/ml	12 ul/ml	12 ul/ml	15 mg/ml	125 ul/ml	(*)
31	-	-	-	-	-	-	+
31	-	-	-	-	-	-	+
40	-	-	-	-	-	-	+
						(*)	
47	-	-	-	-	-	+	+
48	-	-	-	-	-	+	+
49	-	-	-	-	-	+	+
50	-	-	-	-	-	+	+
61	-	-	-	-	-	+	+
					30 mg/ml		
65	-	-	-	-	-	+	+
67	-	-	-	-	-	+	+
70	-	-	-	-	-	+	+
70	-	-	-	-	-	+	+
72	-	-	-	-	-	+	+
76	-	-	-	-	-	+	+
76	-	-	-	-	-	+	+
76	-	-	-	-	-	+	+
76	-	-	-	-	-	+	+
77	-	-	-	-	-	+	+
77	-	-	-	-	-	+	+
78	-	-	-	-	-	+	+
78	-	-	-	-	-	+	+
80	-	-	-	-	-	+	+

Table 7 (Continued)

Estimated Drug Concentration (ng/ml)	NaCl	Visine	Soap	Bleach Drano	Gold Seal	Vinegar	Lemon Juice
	50 mg/ml						
80	-	-	-	-	-	+	+
80	-	-	-	-	-	+	+
80	-	-	-	-	-	+	+
80	-	-	-	-	-	+	+
82	-	-	-	-	-	+	+
82	-	-	-	-	-	+	+
83	-	-	-	-	-	+	+
84	-	-	-	-	-	+	+
84	-	-	-	-	-	+	+
84	-	-	-	-	-	+	+
84	-	-	-	-	-	+	+
89	-	-	-	-	-	+	+
91	-	-	-	-	-	+	+
91	-	-	-	-	-	+	+
91	-	-	-	-	-	+	+
91	-	-	-	-	-	+	+
92	-	-	-	-	-	+	+
96	-	-	-	-	-	+	+
102	-	-	-	-	-	+	+
122	-	-	-	-	-	+	+

(*) No effective interferent concentration found
\- False negative EIA result
\+ Positive EIA result

concentrations of 125 ul/ml of saline would produce negative results in estimated THC concentrations of up to 40 ng/ml. The only interferent being investigated that had no effective concentration to change the positive results to falsely negative was the lemon juice.

Urinalysis Results Evaluation

An initial routine urinalysis was performed on each of the unadulterated 222 GC/MS-confirmed positive specimens used in this investigation and a repeat urinalysis was performed on the adulterated urines that were successful in changing the positive urines to falsely negative results. The urinalysis included determination of the pH, specific gravity, urine color and turbidity. The adulterated urines were composed of a 1:1 mixture of unadulterated urine and interferent. The two sets of urinalysis data were then compared to identify any significant differences. Table 8 summarizes the differences between the adulterated and unadulterated (normal drug free) urine.

It was found that urines adulterated with NaCl had a specific gravity that was completely off the scale of the refractometer (value >1.035). Urines adulterated with bleach, Drano, or liquid handsoap had a high alkaline pH. Conversely, urines adulterated with vinegar had an acidic pH. Urines adulterated with liquid handsoap in a concentration high enough to adversely affect the EIA assay appeared to show abnormal cloudiness. The urines adulterated with golden seal tea had an abnormal brownish

Table 8

Urinalysis Results of Adulterated EIA Positive and GC/MS-confirmed Positive Urines Reported as Falsely Negative

	pH	Specific Gravity	Appearance
Normal Human Urine	5-7	1.005-1.030	clear/yellow
URINE RESULTS AFTER ADULTERATION WITH:			
NaCl			
25 mg/ml	5.5	>1.035	clear/yellow
50 mg/ml	5.5	>1.035	clear/yellow
75 mg/ml	5.5	>1.035	clear/yellow
Liquid Drano			
12 ul/ml	6	1.018	clear/yellow
23 ul/ml	7	1.019	clear/yellow
42 ul/ml	8	1.020	clear/yellow
58 ul/ml	8.5	1.022	clear/yellow
125 ul/ml	11	1.028	clear/yellow
Liquid Handsoap			
12 ul/ml	6	1.018	cloudy/yellow
23 ul/ml	6	1.020	cloudy/yellow
42 ul/ml	7	1.021	cloudy/yellow
107 ul/ml	8	1.033	cloudy/yellow
Visine			
107 ul/ml	5	1.016	clear/yellow
125 ul/ml	5.5	1.018	clear/yellow
Vinegar			
125 ul/ml	4	1.018	clear/yellow
Golden Seal			
15 mg/ml	6	1.022	clear/brown
30 mg/ml	6	1.024	clear/brown

color. It was interesting to note that the only interferent that gave results physiologically similar to normal urine and thus could not be detected through a routine urinalysis was Visine. This was normal and completely expected as Visine is an isotonic solution.

DISCUSSION

After performing EIA assays on the drug-spiked urines and the 222 adulterated GC/MS-confirmed positive specimens, four important observations can be made. First, urine specimens can be adulterated to produce false negative results. In vitro addition of interferents such as NaCl, bleach, Drano, liquid handsoap, Visine, golden seal tea, or vinegar can produce a false negative result in urine containing illicit drug metabolites. Second, concentrations of interferents required to produce false negative results vary with drug concentration and are different for the adulterated GC/MS-confirmed positive urine samples than for the drug-free urine samples spiked with pure drug metabolites. This supports the possibility that interference results from a direct reaction between the drugs or metabolites. This is especially evident when bleach or Drano is added. At specific concentrations, either of these two interferents successfully creates false negative drug results up to a certain estimated drug concentration. To continue to create false negative results with higher drug concentrations, larger quantities of interferent are required. This could also be explained by the oxidation of NADH until the oxidizing capacity of the interferent is used up and then NADH could accumulate and the results turn

positive. Third, consistent results are obtained with increasing concentrations of drugs. There is not a mixture of positive results among the falsely negative results. Finally, the interferents do not effect all of the six drugs assayed in the same manner or in the same concentrations. Table 9 summarizes interferents which caused false negative results. Interference seems to be due to the uniqueness of each drug's chemical and physical properties.

The concentration of interferent required to change the assay results from positive to negative depends on the drug concentration as illustrated in Tables 2 through 7. The THC assay was the assay most easily manipulated to produce false negative results, while the barbiturate assay was the most difficult.

Three criteria were used in selecting possible interferents. First, the change in assay results from positive to negative must not be due to dilution alone. The experimental design called for the assaying of positive urine specimens diluted 1:1 with normal saline to verify that the diluted specimens remained positive. Secondly, quantities of interferent to be added must be small enough to be hidden on one's person. If illicit drug users intended to adulterate their urine for the purpose of avoiding detection, they must conspicuously transport the interferent into the restroom. Thirdly, the added interferent could not leave an obvious precipitate or residue in the urine specimen container.

Table 9

Summary of Interferent Concentrations which Produce False Negative Results in EIA Drug Assays

Assay	Interferent Concentration	Drug Concentration
Amphetamine	75 mg/ml NaCl	0.34-1.42 ug/ml
	12 ul/ml Drano or bleach	0.34-0.52 ug/ml
	23 ul/ml Drano or bleach	0.34-1.80 ug/ml
	42 ul/ml Drano or bleach	0.34-4.50 ug/ml
	125 ul/ml Drano or bleach	0.34-4.65 ug/ml
Barbiturate	75 mg/ml NaCl	0.38 ug/ml
	23 ul/ml liquid soap	0.38 ug/ml
	107 ul/ml liquid soap	0.38-1.10 ug/ml
	23 ul/ml Drano or bleach	0.38 ug/ml
	125 ul/ml Drano or bleach	0.38-1.10 ug/ml
Benzodiazepine	107 ul/ml Visine	0.38-0.78 ug/ml
	42 ul/ml liquid soap	0.38-6.20 ug/ml
	125 ul/ml Drano or bleach	0.38-2.56 ug/ml
Cocaine	75 mg/ml NaCl	0.30-1.18 ug/ml
	42 ul/ml Drano or bleach	0.30-1.18 ug/ml
	58 ul/ml Drano or bleach	0.30-1.72 ug/ml
	125 ul/ml Drano or bleach	0.30-1.82 ug/ml
Opiates	50 mg/ml NaCl	0.31-0.78 ug/ml
	23 ul/ml Drano or bleach	0.31-2.36 ug/ml
	42 ul/ml Drano or bleach	0.31-2.70 ug/ml
	125 ul/ml Drano or bleach	0.31-2.70 ug/ml
Marijuana	25 mg/ml NaCl	31-80 ng/ml
	50 mg/ml NaCl	31-122 ng/ml
	125 ul/ml Visine	31-122 ng/ml
	12 ul/ml liquid soap	31-122 ng/ml
	12 ul/ml Drano or bleach	31-122 ng/ml
	15 mg/ml golden seal	31-61 ng/ml
	30 mg/ml golden seal	31-122 ng/ml
	125 ul/ml vinegar	31-40 ng/ml

The usual volume of urine required for submission to a drug testing laboratory is 60 ml. Based upon a 60 ml urine volume, the minimum amounts of interferent required to change the positive results to falsely negative were calculated. Liquid interferents varied from 0.7 ml to 7.5 ml. The amount of solid interferents required varied from 0.9 grams to 4.5 grams. However, the quantities of interferents required to alter drug testing results depends on drug and metabolite concentrations and purities. Individuals intent on adulterating their urine specimen do not know the drug concentration in their urine.

A determination of the mechanisms by which the interferents are able to alter drug testing results is beyond the scope of this project. However, the evidence suggests that several mechanisms may be involved. NaCl interference suggests ionic strength may alter protein structures as a possible mechanism for altering drug binding or enzyme activities. Also, the high salt concentration could cause the drug to precipitate. The salt could react with the cofactor or substrate and thus interfere with the assay reaction. Vinegar (5% acetic acid), due to its acidic pH, could slow the assay reaction. Liquid bleach (5.26% sodium hypochlorite) and Drano (1.7% NaOH and 6% sodium hypochlorite) are presumed to affect the drug assays by their oxidation capabilities. When liquid bleach or Drano was added to NADH, the NADH was oxidized, decreasing the absorbance at 340 nm. The alkaline pH is

also capable of altering the structure of the enzyme used in the assay. The liquid handsoap (which contains sodium lipid salts) may interfere by a combination of pH and ionic strength or may remove the drug by forming an insoluble complex. Soaps may also increase drug binding sites on the antibody resulting in decreased activity in the assay reaction. Turbidity of the adulterated urine sample may also interfere with absorbance measurements. With golden seal, the active ingredients are claimed to be hydrastine and, to a lesser extent, bereberine; either might compete with the drug for the assay materials. The golden seal tea also altered the 340 nm absorbance of the reaction mixture. The active ingredient in Visine is tetrahydrozaline. With no pH, ionic strengh, or tubidimetric differences from urine, a possible mechanism of interference could be competitive binding for the drug assay materials.

Since it has been shown that the EIA drug assays can be invalidated by specimen adulteration, it is recommended that drug testing should include the assesment of pH, specific gravity, and urine appearance. Suspect urine specimens should be rejected and new specimens obtained. Because urine specimens can be successfully adulterated and not all adulterants can be detected, observed collection is strongly recommended.

REFERENCES

1. Reisch MS. Major chemical producers toughen stance on drug abuse. Clinical and Engineering News 1987:7-12.

2. U.S. Department of Health and Human Services. Urine testing for drugs of abuse. National Institute on Drug Abuse Monogrph 73, 1986:v.

3. Hoyt DW. Drug testing in the workplace--are methods legally defensible. JAMA 1987;258:504-509.

4. Drug testing in major U.S. corporations: A survey of the Fortune 500. Raleigh, NC: Noel Dunviant and Associates, 1985.

5. Allen LV. Specificity of the EMIT drug abuse urine assay methods. Clinical Toxicology 1981;18:1043-1065.

6. Moyer TP. Marijuana testing--how good is it? Mayo Clin Proc 1987;62:413-417.

7. Schwartz RH. Laboratory detection of marijuana use. JAMA 1985;254:788-790.

8. Anonymous. Sure-fire method. Solicited from advertisement in High Times magazine, 1987:78.

9. Duc VT. EMIT tests for drugs of abuse: Interference by liquid soap preparations. Clin Chem 1985;31:658-659.

10. Kim HJ. Interferences by NaCl with the EMIT method of analysis for drugs of abuse. Clin Chem 1976;22:1935-1936.

11. EMIT d.a.u. assay package insert, Syva Co., 1984.

12. Lambert PA. Detection of sample contamination of urine submitted for drugs of abuse testing in a high volume setting using the Olympus 5000 Analyzer. Clin Chem 1987;33:977.